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Mechanisms of action involved in chemically induced effects on male reproductive health

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Background

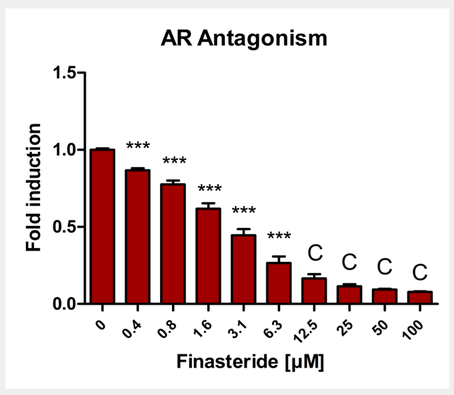
Fetal exposure to anti-androgenic compounds can adversely affect development of the reproductive system and in turn, reproductive function later in life. A shorter anogenital distance (AGD) in newborn animals and humans is a noninvasive biomarker of compromised male reproductive development and health. Although this morphometric effect is thought to be primarily caused by disrupted androgen signaling, the molecular mechanisms underpinning the effect are not well understood. This hinders the development of predictive, non-animal test methods for chemicals.

Aim

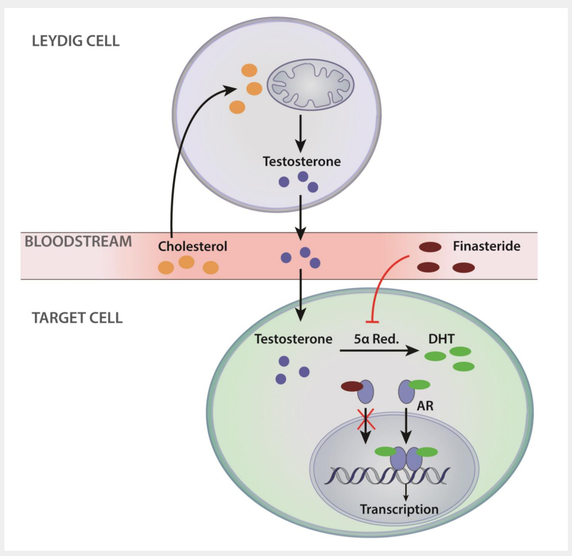
To characterize the molecular mechanisms affected when fetal perineal development is disrupted by xenobiotics.

1. Finasteride is an AR antagonist in vitro

Finasteride is a known 5α-reductase inhibitor. Using an androgen receptor (AR) gene reporter assay, we also find Finasteride to be a strong AR antagonist in vitro.



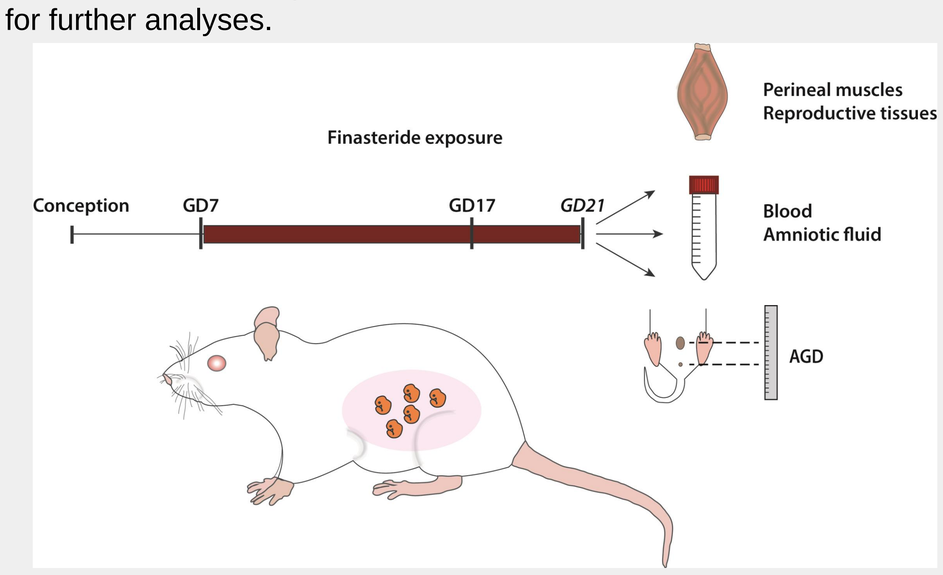
n = 3. One-way ANOVA , Dunnett's post hoc test. ***p< 0.0001. Error bars, mean ± SD



AR: Androgen rector. DHT: Dihydrotestosterone
5α Red.: 5α reductase

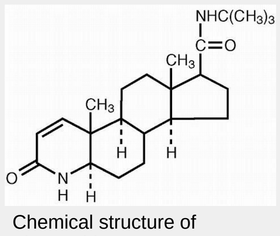
2. In vivo study design

Finasteride (10 mg/kg bw/day) was used to induce shorter AGD in male offspring. Pregnant Sprague Dawley rats were exposed orally during GD7-21. Fetal AGD was measured on GD21 using a stereomicroscope. Blood and amniotic fluids and reproductive tissues were collected at GD17 and GD21 for further analyses.

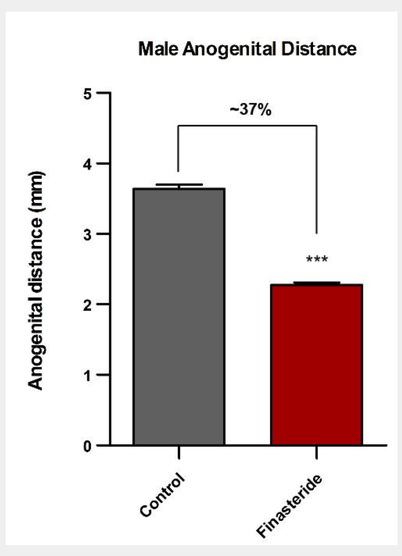


3. Finasteride exposure shortens male AGD

Exposed male fetuses had significantly shorter AGD (~37%) compared to controls at GD21. Results are in agreement with previous findings for Finasteride, and confirms its utility for studying the underlying molecular mechanisms.



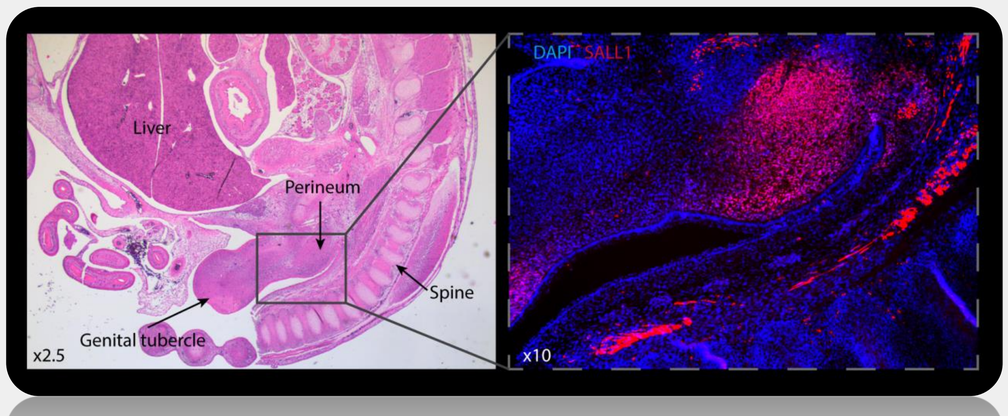
Chemical structure of Finasteride



Results are shown as millimetre ± SEM, n=5-6 litters/group. Statistical significance indicated by ***p< 0.0001. Statistical analyses was adjusted using litter as an independent, random and nested factor. Body weight was used as a covariate. ANOVA, followed by Dunnett's post hoc test in SAS. Female control AGDmm=1.81 mm

4. Molecular profiling in perineal development

Serial sagittal section of GD17 male fetus. Eosin staining (left) for identification of tissues of interest, including perineum. The mesenchymal cell marker SALL1 is expressed in the perineum (right; red). Together with antibodies such as MyoG this will be used to investigate cell type specific expression of the AR and its associated signalling pathways within the perineum, both in normal and disrupted development.



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